



DEPARTMENT OF HEALTH & HUMAN SERVICES

Memorandum

Date: 10 October 2008

From: Consumer Safety Officer, Division of Seafood Safety, HFS-325

Subject: Laboratory Evaluations, Maine Shellfish Sanitation Program

To: Shellfish Specialist, Northeast Region, HFR-NE27

On May 5, 2008 and May 7, 2008, Maine's Department of Marine Resources Marine Biotoxin Laboratories in Lamoine and Boothbay Harbor were evaluated to determine their respective capabilities to continue to support the marine biotoxin portion of Maine's Shellfish Sanitation Program. Accordingly a number of operational and performance records were reviewed prior to the evaluations and together with the findings of the onsite evaluations were used to assess their ability to meet the requirements of the National Shellfish Sanitation Program (NSSP) as established in the *Guide for the Control of Molluscan Shellfish*. Both Laboratories were found to be **in conformity** with these requirements. Conformance to NSSP requirements is necessary to demonstrate the laboratory's capability to continue analytical support to a state's Shellfish Sanitation Program. The Lamoine and Boothbay Harbor Marine Biotoxin Laboratories having been found to conform to NSSP requirements have as a result proven themselves capable to continue to provide biotoxin analytical support to Maine's Shellfish Sanitation Program.

Since the last evaluation, these Laboratories have undergone a number of substantial changes, the most profound of which was the almost complete change in personnel from the top down. Rather than undermining the level and quality of support provided, the Laboratories emerged more capable, efficient and effective in their biotoxin monitoring efforts thanks to the leadership and dedication of the personnel.

The evaluation of each Laboratory was conducted in accordance with Chapter III of the NSSP *Guide for the Control of Molluscan Shellfish*, 2005 Revision. No serious problems were noted, only minor tweaks in their operations are necessary and have been recommended in the evaluation report. The hospitality and cooperation shown by each Laboratory is acknowledged and appreciated. If you have any questions or concerns about these evaluations or the content of this report, please let me know.


Linda A. Chandler

Enclosures

Evaluation Report

On May 5, 2008 and May 7, 2008, Maine's Department of Marine Resources Marine Biotoxin Laboratories in Lamoine and Boothbay Harbor were each evaluated to determine their respective capabilities to continue to support the marine biotoxin portion of Maine's Shellfish Sanitation Program. Accordingly, a number of operational and performance records were reviewed prior to the evaluations and together with the findings of the onsite evaluations were used to assess each Laboratory's ability to meet National Shellfish Sanitation Program (NSSP) requirements as established in the *Guide for the Control of Molluscan Shellfish*. Both Laboratories were found to meet NSSP requirements securing for them the laboratory status of **conforming**. Conforming status is required for a laboratory to continue to provide analytical support to a state Shellfish Sanitation Program. As a consequence of the Lamoine and Boothbay Harbor Biotoxin Laboratories having been found to be in conformity with NSSP requirements, they have proven themselves to be capable of continuing to support Maine's marine biotoxin efforts as part of their Shellfish Sanitation Program.

The staffs of each Laboratory are small but individually and collectively they perform a large number of toxin assays both for monitoring and research purposes. This is made possible by the effective management strategy implemented and the skill and dedication of the staffs of both Laboratories. The combined effect of their efforts is to produce an exceptional program of public health protection. By Laboratory, the Lamoine staff includes Alexandra Rohrer, Scientist and Nicole DeLisle, Conservation Aide. The staff of the Boothbay Harbor Laboratory includes Darcie Couture, Director of the Biotoxin Monitoring Program and Manager of both Laboratories and Laurie Bean, Marine Resources Scientist I.

Since the last evaluation a laboratory conversion factor (CF) value has been established and its relevance to current testing verified weekly or with each set of samples assayed by the Laboratories. The value of a reliable CF value cannot be overstated as a continually functional CF value is necessary to allow the response of the mice to the toxin to be quantified in terms of micrograms of PSP toxin per gram of sample. The quality assurance plan and associated SOPs have been updated and rewritten to promote clarity in content and to foster an improved understanding of what is required. In addition to these substantive changes, there has been an almost complete change in personnel in both Laboratories the result of which is that the Laboratories have emerged more capable, efficient and effective in their biotoxin monitoring efforts.

The evaluations of both Laboratories were conducted in accordance with Chapter III of the NSSP *Guide for the Control of Molluscan Shellfish*, 2005 Revision. No nonconformities were found in either Laboratory. Details of the evaluation are presented below.

Quality Assurance

Both Biotoxin Laboratories operate under the same written quality assurance (QA) plan. This QA plan is basically sound and effectively implemented requiring only minor fine-tuning to enhance its functionality. Suggested tweaks to its content are provided below.

- Page 5, fourth paragraph, recommend changing NSSP Laboratory Certification Program to NSSP Laboratory Program as laboratories are not certified by FDA but merely evaluated and their performance measured against the laboratory requirements set forth by the NSSP.
- Page 14, #6, in the data that was provided for pre-evaluation review, there appears to be some confusion as to how the “median mouse” is determined. The “median mouse” is not the mouse having the median death time of the group of mice injected. The “median mouse” is essentially the median corrected mouse unit of the group of mice injected. Accordingly the “median mouse” is determined by multiplying the death time of each mouse injected in mouse units by its weight correction in mouse units. This gives the corrected mouse unit (CMU) for each mouse otherwise known as the true death time of the mouse. The corrected mouse unit or CMU for each mouse is arranged in ascending order and the median value of the array determined. This value is the corrected mouse unit (MCMU) or the “median mouse”. To clarify the concept of the “median mouse”, it is suggested that #6 be rephrased such that if the median corrected mouse unit/MCMU is greater than 1.92 an appropriate dilution of the sample extract must be made.
- Page 14 under “Dilutions”, first paragraph, suggest rephrasing such that “For any sample in which the median corrected mouse unit/MCMU is greater than 1.92, an appropriate dilution must be prepared and the sample extract assayed again until the median corrected mouse unit/MCMU is between 1.39 and 1.92.
- Page 14 under “Dilutions”, third paragraph, mice in the weight range of 17-23 grams can be used just as effectively as those weighing 19-21 grams as the outcome of the dilution used is based on the median corrected mouse unit/ MCMU which is a function of the death time and a correction for weight. Also suggest that the third sentence of the paragraph be rephrased such that “If the dilution was not dilute enough to produce a median corrected mouse unit/MCMU between 1.39 and 1.92 then a fresh dilution should be prepared with the next ratio on the Dilution Table list. If the dilution was too dilute resulting in a median corrected mouse unit/MCMU of less than 1.39 then a fresh dilution should be prepared with the prior ratio on the Dilution Table list”.
- Page 21 under “Thermometers” in the last sentence, the National Bureau of Standards (NBS) is now the National Institute of Standards and Technology (NIST). Consequently the reference to this organization should be changed to reflect its current name.
- Page 23, #4 under “CF Check Calculation” for clarity, suggest that this be rephrased such that it indicates that the MCMU so determined is the true

median death time of the group. This value is converted to units of time using Sommer's Table. This is the true median death time of the group. It should be between 300 and 420 seconds. If it is not, a different dilution must be used. One which produces a median death time for the group that falls between 300 and 420 seconds.

- Page 23, #5 under "CF Check Calculation" for clarity suggest rephrasing this paragraph to "Once a median death time for the group is found to be between 300 and 420 seconds calculate the Conversion Factor as follows:.....".
- Page 23, #7 under "CF Check Calculation" for clarity suggest rephrasing to "If the CF check was not verified, then five additional mice should be injected with the same dilution that caused the CF check to fail to make a group of ten mice. Another group of ten mice should also be injected with this same dilution to make a second group of ten mice. The CF for each group of ten"

Work Area

The work areas in both Laboratories are clean and well lighted. In the Boothbay Harbor Laboratory work and storage space is ample. In the Lamoine Laboratory, however, work and storage space is more limited but appears to be adequate to support the current level of activity. Mice are adequately accommodated in both Laboratories so as to be fully acclimated before injection.

Equipment

Maintenance of an appropriate pH in the sample homogenate and extract is an essential part of the PSP assay procedure. Rather than use a pH meter to determine and adjust pH, both Laboratories have opted to use pH paper. To achieve accuracy, two pH papers with limited but overlapping pH ranges are used. This eliminates ambiguity in matching color to pH values and improves the minimum accuracy of the pH determination that is achievable.

The balances used in each Laboratory provide the required level of sensitivity. Balance checks are performed monthly using ASTM Class I certified weights. These weight checks are recorded and the records maintained as part of the Laboratory's quality system.

Refrigerator temperature is monitored at the required frequency by both Laboratories. The temperature is maintained in the range of 0-4°C. Temperatures found to be out of limits are adjusted; the refrigerator repaired if persistent; or replaced when necessary which was the case recently in the Lamoine Laboratory. The results of these temperature checks are recorded and the records maintained. Freezer temperatures are also monitored at the required frequency and these results are recorded and maintained as part of each Laboratory's operational record.

Reagent and Reference Solution Preparation and Storage

All glassware/labware used in the preparation of reagents, reference solutions, sample homogenates, extracts and extract dilutions is clean. Reusable glassware/labware is also detergent residue free as determined through testing with bromothymol blue solution (BTB). BTB solution is a pH indicator and as detergents are generally alkaline in nature, BTB is able to detect detergent residues by a change in color. As PSP toxin is especially pH sensitive, the presence of alkaline detergent residues on toxin contact surfaces could adversely impact the assay.

In both Laboratories the PSP standard solution is stored under refrigeration unopened until needed. Once opened, virtually the entire contents, one (1) ml of the 1.2 ml vials is used to prepare the reference solution. The toxin standard remaining is appropriately discarded never stored for future use. The reference solution and the various working dilutions of the reference solution are prepared gravimetrically for accuracy using dilute HCl acid/pH 3 water. The acid solutions used to dilute and extract PSP toxins are prepared from concentrated HCl with good quality laboratory water, free of residual chlorine, the heavy metals of concern and excessive bacterial contamination. PSP reference solution is stored under refrigeration for up to six months. Any remaining after six months is appropriately discarded. Refrigerated, stored PSP reference solution is weighed before aliquots are withdrawn for further use to alert the staff to potential inadvertent changes in the toxin concentration caused by evaporation. Working dilutions of the reference solution are not stored but immediately discarded after use.

Collection and Transportation of Samples

Samples are collected in clean, waterproof, puncture resistant plastic bags which are appropriately labeled with the shellfish type, collectors name, harvest area and date and time of collection. Samples are placed in an ice chest which is cooled to below 10°C with ice or cold packs for transportation to the laboratory. Samples are collected early in the day so that they can be tested within 24 hours of collection. Those samples which cannot be tested on the day of collection are washed, shucked, drained, extracted, centrifuged and their supernatant decanted and refrigerated for next day bioassay.

Preparation of Sample

At least 12 shellfish are used per sample. The shells of the shellfish are cleaned of external debris, properly opened and the meats carefully removed from the shell so as to minimize damage to the meats. The meats are subsequently rinsed with fresh water and allowed to drain in a single layer on a #10 sieve for five (5) minutes. Drained meats are blended for 60-120 seconds until homogenous in preparation for extraction.

Extraction

One hundred (100) grams of homogenate is weighted into a large beaker and an equal volume by weight of 0.1N HCl is added; the contents stirred to homogeneity and the pH

taken using pH paper in the appropriate pH range for the assay. The pH is adjusted if necessary by the dropwise addition of either 0.1N NaOH if it is below pH 2 or 5N HCl if it is above pH 4 and the mixture brought to a boil ($100\pm 1^{\circ}\text{C}$). Once boiling, the mixture is allowed to boil gently for five (5) minutes. After boiling, the extract is cooled to ambient temperature, the pH taken and adjusted if necessary as indicated above. The final volume is subsequently determined and adjusted by weight to 200 grams (the original volume of acidified sample homogenate) with pH 3 water to compensate for boiling loss. The extract is stirred to homogeneity and an aliquot poured into a centrifuge tube; centrifuged at 3000 rpm for five (5) minutes and the supernatant collected in preparation for the bioassay.

Bioassay

The bioassay procedure as demonstrated by both Laboratories is consistent with the requirements of the NSSP. The mice used by both Laboratories are fully acclimated and only those mice in the appropriate weight range (17-23 grams) are used for the bioassay. Injections are 1 ml in quantity introduced intraperitoneally by 26 gauge syringe. Death time is recorded to the nearest second and indicated by the last gasping breath. Mice are observed continuously for up to 20 minutes. A minimum of three (3) mice are used per sample. Dilutions are made when the median death time of the group of mice injected is less than 300 seconds (1.92 mouse units). Dilutions are made using pH 3 water as the diluent to maintain the sample extract within the appropriate pH range of the assay and are designed to produce a median death time of 300 to 420 seconds (1.39 to 1.92 mouse units) within the group of mice injected. A weight correction in mouse units is applied to each mouse injected so that its true death time can be calculated.

A routine conversion factor check is performed once per week during the PSP season by both Laboratories. There was some confusion as to the appropriate dilution to be used in checking the conversion factor (CF) value. After some discussion the problem appears to have been resolved. The guiding principle in checking the CF is to use a dilution which produces a median death time within the 5 to 7 minute (300 to 420 second) range. This requirement has been written into the SOPs to stress its importance and to ensure compliance.

Five (5) mice are used for the routine CF check. The routine CF check when properly performed should give values within $\pm 20\%$ of the Laboratories' established CF value of 0.21. This was the case for both Laboratories. When the 5 to 7 minute (300 to 420 second) death time rule was adhered to, neither Laboratory failed to verify the established CF value demonstrating the continued relevance of the 0.21 value in estimating sample toxicity.

Calculation of Toxicity

Calculation of sample toxicity by both Laboratories is consistent with the requirements of the NSSP. A weight correction in mouse units is applied to each mouse injected as indicated above. The death time in seconds is converted to mouse units. A value of

<0.960 or <0.875 mouse units is assigned to survivors depending upon the length of the period of observation, <0.960 for a 1200 second (20 minute) observation time and <0.875 for a 3600 second (60 minute) observation time. The true death time for each mouse injected is calculated by multiplying the weight correction in mouse units by the death time in mouse units. The resulting values are arrayed in ascending order and the median value, the median corrected mouse unit (MCMU) determined. The MCMU is multiplied by the established CF value of 0.21, the dilution factor if any and 200, the original value of the acidified sample extracted. Toxicity is calculated as micrograms of PSP toxin per 100 grams of sample.